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(54) Title: NUCLEOZYMES

(57) Abstract

Nucleozymes containing ribonucleotides and deoxyribonucleotides or nucleic acid analogues are described herein. The nucleozymes have catalytic activity and are significantly more resistant to degradation than their all-RNA ribozyme counterparts. Also described are methods for preparing the nucleozymes along with methods of using nucleozymes, e.g., as therapeutic agents.

NUCLEOZYMES

Field of the Invention

This invention relates to nucleozymes, mixed nucleic acid polymers having catalytic activity. The invention also relates to methods of preparing and using nucleozymes.

Background of the Invention

Proteins were the only known catalysts of cellular reactions until the discovery of RNA catalysts (ribozymes). In some instances, the folded structure of a ribozyme catalyses a cleavage reaction on another part of the same molecule (cis-reaction). In other instances (trans-reaction), the ribozyme may also act as a catalyst on another RNA or DNA molecule (substrate) by cleaving or ligating pieces of the substrate without changing itself in the process. (Zaug et al. Science, Vol. 231, pp. 470-75, 1986; Cech Science, Vol. 236, pp. 1532-39, 1987).

A well-characterized example of a ribozyme is the self-splicing Group I intron from the nuclear rRNA of Tetrahymena thermophila. An intron is an intervening sequence in a eukaryotic gene which does not encode a protein or in rare cases encodes a different protein. Introns are transcribed along with coding sequences (exons) to produce precursor RNA. The introns are removed from the precursor RNA and the exons are ligated by RNA cleaving and splicing steps. The Group I intron or ribozyme of T. thermophila catalyzes its own removal from the precursor RNA molecule. (Kruger et al. Cell

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31:147-157, (1982); Zaug et al. (1986)). The
self-splicing ribozyme catalyzes a variety of
phosphodiester transfer reactions. The ribozyme can
act as a ribonuclease, ligase, phosphotransferase,
acid phosphatase, polymerase and RNA restriction
endonuclease (Zaug, A.J., et al , Science 231:470-475
(1986); Zaug, A.J., et al., Nature 324:429-433
(1986); Zaug, A.J., et al., Biochemistry 25:4478-4482
(1986); Been, M.D., et al., Science 239:1412-1416
(1988); Doudna et al., Nature 339:519-522 (1989); all
incorporated by reference herein).

The "hammerhead" and "hairpin" ribozymes also
have been studied and described (Perreault et al.,
Nature 344:565-567 (1990); Perreault et al.
Biochemistry 30:4020-25 (1991); Yang et al.
Biochemistry 29:11156-60 (1990); Chowrira et al.
Biochemistry 30:8518-22 (1991); Uhlenbeck Nature,
328:596-600 (1987)). The hammerhead ribozyme forms a
stem loop secondary structure to form the
catalytically active molecule. The hairpin ribozyme
has a structure resembling a hairpin.

Although ribozymes are intriguing molecules,
their use for in vivo applications is limited if not
precluded. The all-RNA molecules are susceptible to
degradation from enzymes (RNAses) present in vivo.
There presently is no way known to inventors for
delivering such molecules to the intended site in an
active form.

Summary of the Invention

The present invention is based on the discovery
that ribozymes have catalytically critical sites and
that it is not necessary to have an all-RNA molecule
to have catalytic activity. The ribozyme like
molecules of the invention or "nucleozymes" have
ribonucleotides or nucleic acid analogues
(hereinafter NAAs) at catalytically critical sites
and NAAs or deoxyribonucleotides at non-catalytically
critical sites. The preferred nucleozymes have
ribonucleotides at catalytically critical sites.
Nucleozymes have catalytic activity on the same
substrates as their ribozyme counterparts.

The nucleozymes of the present invention thus
essentially are modified ribozymes having at least a
portion, or all, of the ribonucleotides replaced with
deoxyribonucleotides or NAAs. The nucleozymes are
significantly more resistant to degradation than
their all-RNA ribozyme counterparts because the
chemicals or enzymes present in vivo do not recognize
the nucleic acid internucleotide bonds. The
resistance can be to either enzymatic or chemical
degradation. Preferably, a majority of the
ribonucleotides of the ribozyme are replaced with
deoxyribonucleotides or NAAs. The stability of the
nucleozymes allows them to be useful as therapeutic
agents whereas ribozymes would be cleaved and
rendered inactive by enzymes, e.g. RNAses, present in
vivo.

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The nucleozymes of the present invention are
chimeric nucleic acid polymers having catalytic
activity due to and preferably optimized by the
presence of RNA or a NAA at a catalytically critical
site. The present invention provides chemistry which
allows synthesis of the chimeric polymers and the
determination of catalytically critical sites. The
sites may be determined by varying the location of
deoxyribonucleotides in a chimeric polymer and
determining the locations responsible for or related
to the chimeric polymer's ability to catalyze.

The present invention also pertains to a method
for making a chimeric polymer. The polymers are made
by phosphitylating protected ribonucleotides or NAAs
units under conditions to form substantially
pure-protected phosphoramidites or synthons of a
single isomer. The protected phosphoramidites are
coupled to each other forming a protected chimeric
nucleic acid chain. The protecting groups are
removed from the chimeric nucleic acid chain under
conditions which completely deprotect the polymer.

The method of the present invention alleviates
problems associated with a known prior art method for
preparing chimeric RNA/DNA polymers (Perreault et al.
Nature 344:565-567 (1990); Wu et al. Journal of the
American Chemical Society 111:8531-33 (1989)). The
prior art synthetic method for making chimeric
polymers had problems with the migration of the
protecting groups during the phosphitylating step,
difficulty in removing the protecting groups and also

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has problems resulting from the process of removing
the protecting groups in the deprotection step. The
former problem results in the production of monomer
units having protecting groups in an undesired
position. The latter problem results, in many cases,
in a) nucleotide modification, b) phosphodiester
linkage isomerization, and c) to retention of a
substantial amount of protecting groups on the
polymer resulting in a non-functional polymer. The
first problem was overcome in the present invention
by selecting a catalyst capable of minimizing
migration of protecting groups, e.g., a combination
of 2,4,6-collidine and N-methylimidazole. The
problem in removing the protecting groups was
eliminated by deprotecting the mixed polymer in the
presence of ethanolic ammonia.

The present invention also pertains to methods
of using the nucleozymes. The nucleozymes may be
used to perform the same catalytic functions as their
all-RNA ribozyme counterparts. For example, a
nucleozyme may be used as a ribonuclease, ligase,
phosphotransferase, acid phosphatase, polymerase, or
an RNA restriction endonuclease. The nucleozymes may
be used to selectively cleave and ligate substrates
by contacting the substrates with a nucleozyme such
that the nucleozyme targets a specific sequence in
the substrate for cleavage or ligation. The
nucleozymes may be used as polymerases to polymerize
the production of an oligoribonucleotide or an
oligodeoxyribonucleotide. The nucleozymes also may
be used in place of antisense RNA technology.

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The nucleozymes also may be used as therapeutic agents introduced in vivo due to their resistance to chemical and enzymatic degradation. The nucleozymes may be used, for example, in a method for treating a subject for a retrovirus associated disease, e.g., human immunodeficiency virus (HIV). The method involves administering a therapeutically effective amount of at least one nucleozyme to the subject such that the nucleozyme cleaves the RNA genome of the retrovirus rendering it inactive. A plurality of nucleozymes also may be administered if it is desirable to target more than one sequence in the RNA genome.

A nucleozyme may be provided in a pharmaceutical composition. The pharmaceutical composition would include at least one nucleozyme and a pharmaceutically acceptable carrier.

It is an object of the present invention to provide a nucleozyme capable of maintaining its catalytic properties in vivo.

It is an object of the present invention to provide a chimeric nucleic acid polymer having catalytic activity.

It is yet another object of the present invention to provide a method for preparing chimeric polymers which are free of protecting groups and undesired isomeric side products.

It is yet another object of the present invention to provide a homogenous chimeric polymer.

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Brief Description of the Drawings

FIG 1 depicts a structure of a hammerhead nucleozyme.

FIG 2 is a table listing nucleozymes of the present invention indicating the positions of ribonucleotides in the nucleozymes. The position designations correspond to the structure depicted in FIG 1.

FIGs 3 and 4 are photographs of a gel demonstrating the cleavage of a $5'-^{32}P$-labeled RNA substrate. The designation "RNA" is the all-RNA containing ribozyme and the nucleozymes are as abbreviated in FIG 2.

FIG 5 is a photograph of a gel demonstrating the cleavage of a radioactive RNA substrate by nucleozymes of the present invention containing methoxy substituted NAAs. The nucleozymes are abbreviated as in FIG 2.

FIGs 6 and 7 are graphs depicting the stability of nucleozymes compared to the stability of the all-RNA ribozyme counterpart after exposure to both RNAse A (6) and a yeast cellular extract (7).

Detailed Description

The nucleozymes of the present invention have catalytic activity. Nucleozymes essentially are modified ribozymes preferably having at least one ribonucleotide or nucleic acid analogue (NAA) at a catalytically critical site(s) and deoxyribonucleotides or NAAs at non-critical sites.

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The term "nucleozyme" is intended to include
catalytic chimeric polymeric chains containing
ribonucleotides and deoxyribonucleotides and/or
nucleic acid analogues.

A ribozyme is an all-RNA containing molecule
capable of being a biological catalyst. Ribozymes
are art recognized and examples of such molecules
include the "hammerhead" or "hairpin" ribozymes.

The term "catalytic activity" is intended to
include many types of catalysis. For example, the
catalytic activity could be that of a ribonuclease,
ligase, phosphotransferase acid, phosphatase,
polymerase, and RNA restriction endonuclease.

The term "nucleic acid analogue" (NAA) is
intended to include analogues which are structurally
similar to ribonucleotides or deoxyribonucleotides
and are capable of being monomer units in a polymer
capable of hybridizing with DNA or RNA. The analogue
may impart properties to a polymeric chain which
differ from those of a nucleotide but the analogue is
capable of being a monomer unit in a polymeric
chain. The NAA may impart resistance to chemical or
enzymatic degradation to the chimeric polymer. NAAs
may be selected for their structural conformation if
a particular conformation is desired for the
polymer. A NAA which is structurally similar to a
ribonucleotide may be positioned at a catalytically
site if the NAA is capable of participating and/or
attaining the desired catalytic activity.
Preferably, if the NAA is positioned at a

catalytically critical site it has a formula as
depicted in Formulas I-IV below wherein X is a good
coordinating ligand with divalent metal ions, eg.,
Mg^{+2}.

A nucleotide analogue may contain a heterocyclic
ring as depicted in the formulae I-IV below or may be
acyclic as shown in Formula V below. The preferred
heterocyclic ring containing analogues of the present
invention have three hydroxy substituents, alkoxy
substituents or combinations thereof.

Formula (I)   Formula (III)
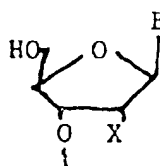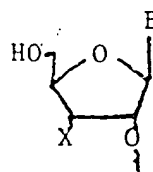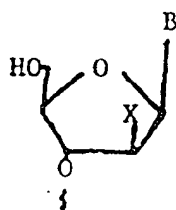
Formula (II)   Formula (V)   Formula (IV)
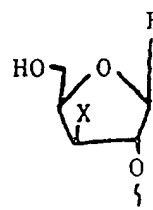
In the above formulae, B is a base. The base may be
substituted or unsubstituted. Examples of bases
include adenine, cytosine, guanine, uracil,

2-aminopurine, hypoxanthine, imidazole, $R_1-\overset{\overset{\textstyle O}{\|}}{C}-O-$,

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R_1-SH-, and R_1-NH$_2$-. The term "base" is
art-recognized and one of ordinary skill in the art
would know which bases are useful in the present
invention. X is selected from the group consisting
of -OR$_1$, F, -R$_2$OH, -NH$_2$, -R$_2$NH$_2$, -Br,
-R$_2$Br and -R$_2$F wherein R$_1$ is a lower alkyl
group and R$_2$-R$_4$ are a lower alkyl hydrocarbon
chains. The term "lower alkyl" is intended to
include from one to six carbons, more preferably one
to three carbons and most preferably a single carbon
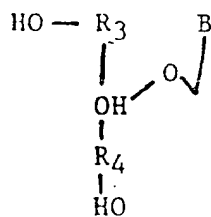atom. For example, the most preferred X is methoxy.

B may be protected during the synthesis
process. The protecting groups may be the
conventional groups typically used in oligonucleotide
synthesis processes e.g., N^6-benzoyl for adenine,
N^4-benzoyl for cytosine, N^2-isobutyryl for
guanine, and N^2-benzoyl for 2-aminopurine. Other
useful protecting groups include phenoxyacetyl (PAC)
and t-butoxyacetyl (TAC). One of ordinary skill in
the art would know which protecting groups are
appropriate for a particular base.

The NAAs capable of being positioned at a
catalytically critical site may be determined by one
of ordinary skill in the art using the following
screening process. The catalytically critical sites
of a particular ribozyme may be determined by
positioning deoxyribonucleotides(s) at various
locations within the ribozyme and evaluating the
chimeric polymer's catalytic activity. After
determining the locations of the catalytically

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critical sites, NAAs may be substituted for the
deoxyribonucleotide(s) and the chimeric polymer's
catalytic activity is again evaluated. If the NAA
containing chimeric polymer possesses catalytic
activity then the NAA is suitable for positioning at
a catalytically critical site.

The term "synthon" is intended to include the
fully protected monomer units (phosphoramidites) used
to assemble the nucleic acid analogues of a chimeric
polymer chain. The term "nucleic acid analogue" is
used to describe the units when polymerized as part
of a chimeric polymer chain.

The term "chimeric polymer" is intended to
include polymers containing at least two different
types of monomer units, e.g., RNA, DNA, or NAA. For
example, a chimeric polymer may include RNA/DNA,
RNA/NAA, or DNA/NAA polymeric chains. It should be
understood that the linkages between the building
units of the polymeric chain may be linkages capable
of bridging the units together for either _in vitro_ or
in vivo. For example, the linkage may be a
phosphorous containing linkage, e.g., phosphodiester
or phosphothioate, or may be a nitrogen containing
linkage, e.g., amide. It should further be
understood that the chimeric polymer may contain
non-nucleotide spacer molecules along with its other
nucleotide or analogue units. Examples of spacer
molecules which may be used are described in Nielsen
et al. _Science_, 254:1497-1500 (1991), the contents of
which are expressly incorporated by reference.

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The analogues described above may be prepared
using synthetic methods capable of attaching the
desired moieties at the selected positions. Other
possible analogues will be apparent to one of
ordinary skill in the art. Deoxyribonucleotides are
readily available and may be purchased from a variety
of sources. The alkyl substituted heterocyclic ring
containing analogues may be synthesized by first
reacting the moiety being modified with arabino
triflates (trifluoromethane sulfonates) and second
displacing the arabino triflates with an appropriate
organotin compound selected on the basis of the
desired moiety. A schematic of this reaction is
depicted below:
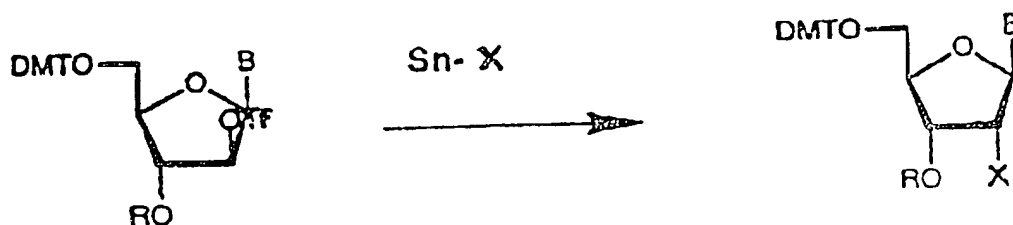
This synthesis scheme may be used to prepare
analogues wherein X is selected from the group
consisting of $-OR_1$, $-R_2OH$, R_2F, $-RBr$, and
$-RNH_2$. One of ordinary skill in the art would know
how to synthesis the halogenated analogues (X is $-F$
or $-Br$) and the amino substituted analogues (X is

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-NH$_2$). These analogues may be synthesized as
described by Williams et al., Biochemistry,
30:4001-4009 (1991), Doerr et al., J. Org. Chem.,
32:1462 (1967), Mengel et al. Angew. Chem., 90:557
(1978), or Coddington et al., J. Org. Chem., 29:558
(1964), the contents of each of the references are
hereby expressly incorporated by reference.

    The acyclic nucleic acid analogues may be
prepared by reacting a protected acyclic molecule
with a diol. Reaction processes which may be used
are described in Durand et al., Nucleic Acid
Research, 18:6353 (1990); Seela et al., Nucleic Acid
Research, 15:3113-3124 (1987); Cload et al., JACF,
113:6324-6326 (1991), the contents of each reference
is hereby expressly incorporated by reference.

    The nucleozymes have catalytically critical
site(s) at which a ribonucleotide or NAA is necessary
for the nucleozyme to have the desired level of
catalytic activity. The term "catalytically critical
site" is intended to include sites which, if altered
from a ribonucleotide or a NAA to a
deoxyribonucleotide, substantially reduces or even
eliminates catalytic activity. A substantial
reduction in catalytic activity would be that
reduction which limits the usefulness of the
nucleozyme as a catalyst in vitro or in vivo. The
catalytically critical sites may be determined for
each nucleozyme. Catalytically critical sites can be
determined by preparing a variety of chimeric
polymers using the chemical techniques described

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herein and comparing the catalytic activity of the
chimeric test polymers. The catalytically critical
sites in the all-RNA containing ribozyme counterparts
are determined by selecting sites believed to be
involved in catalysis and inserting a
deoxyribonucleotide at the particular site. If the
chimeric polymer does not have the same or
substantially the same catalytic activity as the
all-RNA counterpart, then the selected site is
presumed to be a catalytically critical site. The
hammerhead nucleozyme has four catalytically critical
sites which are the G9, G12, A13 and A29 positions
for the sequence depicted in FIG 1.

The nucleozymes of the present invention are of
a size capable of being synthesized using the
chemistry described herein. Preferably, the
nucleozymes have less than about 100 total building
units, more preferably, less than about 80 building
units, even more preferably, less than about 70
building units, and most preferably less than about
50 building units. Some nucleozymes may even have
less than about 20 building units. The preferred
nucleozyme is modeled after the hammerhead ribozyme,
the catalytic portion of which has 35 building
units. The term "building unit" is intended to
include ribonucleotides, deoxyribonucleotides, or
synthons.

The nucleozymes of the present invention are
modified ribozymes having at least a portion of the
ribonucleotides replaced with deoxyribonucleotides or

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NAAs. The modified ribozymes or nucleozymes are
significantly more resistant to degradation than the
all-RNA counterparts. The degradation may be either
enzymatic or chemical degradation. The language
"significantly more resistant to degradation" is that
resistance which allows the nucleozyme to remain
largely intact for an extended period of time
relative to its all-RNA counterpart. Preferably it
has a resistance which allows it to be administered
for in vivo applications.

Resistance to enzymatic degradation may be
resistance to enzymes present in vivo, e.g. RNAses
such as RNAse A. FIGs. 6 and 4B are graphs depicting
the relationship between the percentage of the
nucleozyme being intact at a particular RNAse A (FIG.
6) or yeast cellular extract (FIG. 7) concentration.
The data set forth in FIGS. 6 and 7 was obtained
using the following procedure. A sample of 0.001
pmol of 5' labeled nucleozyme or ribozyme was
incubated with 0.5 mg of carrier TRNA in 50 mM
Tris-HCl (pH 7.4) and 10 mM Mg^{+2} and different
concentrations of RNAse A or yeast extract ten
minutes for A, thirty minutes for extract. The
reactions were stopped by the addition of 20 mM EDTA
and loaded and analyzed on 15% PAGE in 7M urea. The
yeast extract was prepared from a 1 ml culture of
yeast strain BWG2-9A grown to late-lag phase,
harvested and washed with 25 mM sodium phosphate
buffer (pH 7.8). The pellet was suspended in 100
μl of the same buffer and sonicated for 20 seconds

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(60W). After centrifugation for five minutes in an
Eppendorf centrifuge, the supernatant was used
directly after appropriate dilutions and incubations
with the nucleozymes and the all-RNA ribozyme.

As shown in FIG. 6, the nucleozymes were at
least about 75% intact at an RNAse A log
concentration of -1.5 and at least about 80% intact
at an RNAse A log concentration of -2.5. Preferably,
the nucleozyme is at least about 90% intact at both
concentrations. The difference in stability between
the nucleozymes and ribozyme is even more apparent
when the enzyme is a yeast cellular extract (FIG.
7). The nucleozymes were at least 90% intact at all
of the tested concentrations.

The chemical degradation for purposes of this
invention is intended to include resistance to
chemicals present in vivo and in vitro. The
resistance may be to alkaline hydrolysis, e.g.,
sodium hydroxide and water.

The preferred nucleozymes of the present
invention are modified ribozymes having a majority of
the ribonucleotides replaced with
deoxyribonucleotides or NAAs. At least one of the
units is a deoxyribonucleotide and preferably most of
the units are deoxyribonucleotides. The nucleozymes
more preferably, have at least about 75%, even more
preferably at least about 85%, most preferably at
least about 90% of their ribonucleotides replaced
with deoxyribonucleotides or NAAs. The nucleozyme
also may be made up entirely of NAAs or a combination
of NAAs and deoxyribonucleotides.

The present invention also pertains to a method
of making a chimeric polymer. The method is similar
to that described by Scaringe et al. to produce an
all-RNA polymer. (<u>Nucleics Acid Research</u> Vol. 18,
No. 18, 5433-41 (1990)), the contents of which is
expressly incorporated by reference. The method
includes the steps of phosphitylating protected RNA,
DNA or NAA units under conditions which minimize
migration of the protecting group forming
isomerically pure protected phosphoramidites. The
protected RNA or synthons may have the desired
moieties protected with the protecting groups capable
of surviving the phosphitylation and coupling steps.
Examples of such groups include conventional DNA
protecting groups such as 5'-O-DMT, N-Bz (Ade and
Cyt), N-iBu (Gua), ß-cyanoethyl for phosphate, TB DMS
for 2'-hydroxyl.

The term "isomerically pure protected
phosphoramidites" is intended to include
phosphoramidite preparations free of a substantial
amount of undesired isomers of the phosphoramidites.
A substantial amount is that amount which would
substantially interfere or impede with the
preparation's ability to be used in forming a mixed
polymeric chain. For example, a consideration in the
chemical synthesis of a ribonucleotide
phosphoramidite is contamination of the desired
2'-O-protecting group-3'-O phosphoramidite with the
undesired 3'-O- protecting group
-2'-O-phosphoramidite. Syntheses performed with the

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latter lead to oligonucleotides having 5'-2'
linkages. Isomerically pure protected
phosphoramidites of the present invention lead to
oligonucleotides which are free of such undesired
linkages.

The method of the present invention also
involves coupling the protected phosphoramidites
together forming a protected chimeric polymeric
chain. The coupling can be done using well-known
chemical techniques known to one of ordinary skill in
the art. Preferably the coupling is done on an
automated synthesizer.

After the chimeric polymeric chain is formed,
the protecting groups now can be removed from the
chain under conditions which completely deprotect the
polymer. Also, nucleotide base modification and/or
phosphodiester linkage isomerization of the chain now
can be minimized during the deprotection step.

The prevention of migration of the protecting
groups in the phosphitylation step can be
accomplished by phosphitylating in the presence of a
catalyst selected to minimize migration. An example
of such a catalyst is the combination of 2, 4,
6-collidine and N-methylimidazole.

The invention permits removal of the protecting
groups in a manner that is complete and that
minimizes nucleotide base modification and/or
phosphodiester linkage isomerization. Complete
removal of protecting groups includes substantially
complete removal where a polymeric chain may have a

small number of protecting groups still attached
which do not effect the polymeric chain's intended
catalytic function. The deprotection step is
accomplished by deprotecting the polymer in the
presence of an agent capable of minimizing such
effects. An example of such an agent is ethanolic
ammonia.

The method also allows the production of a
homogeneous RNA/DNA polymer free of undesired
isomeric products. The term "free" is intended to
include substantially free wherein a small amount of
protecting groups or undesired isomers are present as
long as the amount does not interfere or impede the
polymer's function. The minimization of nucleotide
base modification is intended to include that
modification which would effect a polymeric chain's
intended catalytic function. Minimizing
phosphodiester linkage isomerization, when used in
connection with a molecule, means preventing that
degree of isomerization which would, adversely affect
the molecule's intended catalytic function.
Minimizing phosphodiester linkage isomerization when
used in connection with a preparation, means
preventing that degree of isomerization which would
substantially affect the preparation's abilities to
be used for its intended catalytic functions.

The present invention also pertains to methods
for using the nucleozymes. The nucleozymes may be
used for any method in which a ribozyme presently may
be used. For example, the nucleozyme may be used to

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selectively cleave an RNA substrate or to ligate two
pieces of RNA together. When cleaving a substrate,
the RNA substrate is contacted with at least one
nucleozyme which targets a specific sequence in the
substrate for cleavage. A plurality of nucleozymes
also may be used in a cleavage process.

The nucleozymes of the present invention also
may be used to polymerize an oligonucleotide
molecule. The oligonucleotide may contain
ribonucleotides, deoxyribonucleotides, and analogues
of deoxy- or ribonucleotides. A template is
contacted with a population of the appropriate
nucleotide monomer units and a nucleozyme under
conditions which allow an oligonucleotide
complementary to the template to form. The template
preferably is attached to a support.

The nucleozymes of the present invention also
may be used for therapeutic methods due to their
stability _in vivo_. The nucleozymes may be used, for
example, to treat a subject for a retrovirus
associated disease. A therapeutically effective
amount of at least one nucleozyme is administered to
a subject such that the nucleozyme(s) cleaves the RNA
genome of the retrovirus or the viral mRNA rendering
it inactive.

A retrovirus associated disease is intended to
include diseases involving retroviruses.
Retroviruses have an RNA genome making them
susceptible to cleavage by the at least one
nucleozyme or the plurality of nucleozymes. An
example of such a retrovirus associated disease is
AIDS wherein the causative retrovirus is the human
immunodeficiency virus (HIV).

The term "subject" is intended to include living
organisms susceptible to retroviruses, e.g.,
mammals. Examples of subjects include humans, cats,
and rats.

The language "therapeutically effective amount"
is intended to include that amount capable of
eliminating or significantly reducing the symptoms
associated with retrovirus associated diseases. The
amount may be determined on an individual basis and
will be based, at least in part, on consideration of
the severity of symptoms to be treated, the results
sought and the size of the subject. Thus, a
therapeutically effective amount may be determined by
one of ordinary skill in the art employing such
factors using no more than routine experimentation.

The nucleozymes of the present invention also
may be used in conjunction with or in place of
antisense RNA technology. That is, to control the
expression of a gene by targeting an appropriate
mRNA. A nucleozyme may be selected based on its
ability to target a particular mRNA sequence and
subsequently an effective amount of the nucleozyme
may be administered to a subject. The effective
amount would be that amount necessary to target the
RNA and control expression of a selected gene.

The present invention also pertains to
pharmaceutical compositions containing at least one
nucleozyme and a pharmaceutically acceptable
carrier. The language pharmaceutically acceptable
carrier is intended to include carriers capable of
being co-administered with the nucleozyme(s) while
not adversely affecting the nucleozyme(s) catalytic

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activity. The carrier may be solid or liquid or a
gel. Examples of liquid carriers include water, an
aqueous solution of a non-toxic salt, e.g., sterile
physiological saline solutions, or aqueous solutions
containing organic solvents, e.g., ethanol. Also
suitable are emulsions, such as oil-in-water. Solid
carriers may include nutritive carriers, e.g.,
sucrose or gelatin, or non-nutritive carriers, e.g.,
cellulose or talc.

The Preferred Embodiment

The preferred nucleozymes of the present
invention are modeled after the "hammerhead"
ribozyme. The moderate size of the catalytic
hammerhead domains of RNA lends itself to chemical
synthesis. As shown in FIG. 1, the conserved
hammerhead domain of around fifty ribonucleotides
found in naturally occurring RNA has been petitioned
between a thirty-five unit catalytic fragment 10, the
ribozyme, and a fourteen nucleotide substrate unit 12.

The all-DNA analog of the hammerhead domain is
inactive in catalysis. It was determined that
nucleozymes containing as few as four ribonucleotides
out of a total of thirty five nucleotides have
catalytic activity. Active hammerhead nucleozymes
require the presence of ribonucleotides in particular
at four positions, particular the G9, G12, A13, and
A29 positions shown in FIG. 2.

FIG. 2 is a table showing the chimeric polymers
prepared along with the positions of ribonucleotides
within the polymers. Nucleozymes are abbreviated as
follows: DR and MR designate nucleozymes composed of

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predominantly deoxyribonucleotides and 2'
-methoxynucleotides, respectively. The following
number indicates the number of ribonucleotides and
the final number refers to a particular combination
of ribonucleotides.

The all DNA analog of the hammerhead domain is
inactive in catalysis. Several RNA/DNA mixed
polymers appear as set forth and designated in the
table of FIG 2. As shown in FIGs 3 and 4, the mixed
polymer having ribonucleotides at positions 9, 10,
12, 13, 28, 29 and 30 of the ribozyme showed good
catalytic activity (FIG 3). The nucleozyme
containing the fewest ribonucleotides, DR4R3 and
DR4R2, showed at least some activity. In FIGs 3 and
4, S indicates the mobility of the intact substrate
and P, the product. RNA is the all-RNA ribozyme and
nucleozymes are abbreviated as in FIG 1. The
presence or absence of the enzymatic fragment is
indicated by + or -, respectively in FIG 3. Lane 1
is a control lacking the added catalytic fragment in
FIG 3. Lane 1 in FIG 4 is the 4.5 hour incubation of
the substrate with neither Mg^{+2} nor nucleozyme and
lane 2 is an incubation control in the presence of
Mg^{+2} but no added nucleozyme. The samples were
analyzed on 15% PAGE in 7M urea.

FIG 3 is a photograph showing the cleavage of
radioactive substrates by OMe-nucleozymes. The S
indicates the mobility of the intact substrate and P,
the product. The reactions were performed as in
FIG 2 except that the incubations of the substrate
were with the MR5R nucleozyme, for four hours and the
(Mg^{+2}) was varied. Lane 1 contained no Mg^{+2},

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lane 2-10 mM, lane 3-20 mM, lane 4-30 mM and lane
5-50 mM. The OMe-nucleozymes are nucleozymes having
synthons which are methoxysubstituted in the 2'
position. The nucleozymes designated MR contain all
methoxysubstituted nucleozymes rather than
deoxyribonucleotides.

The catalytic activity of the nucleozymes was
further evaluated by determining parameters including
K_M and K_{cat} for some of the kinetic reactions.
In addition, the K_{Mg}, K_S and k_3 of nucleozyme
reactions were obtained to evaluate how extensive
deoxyribonucleotide substitution effects Mg^{+2}
cofactor binding. A summary of these results is
presented in Table 1 below.

TABLE I Kinetic parameters of nucleozyme-catalyzed
reactions[a]

Substrate/ Nucleozyme	K_M (μM)	Kcat (1/min)	K_S (μM)	K_3 (1/min)	K_{Mg} (mM)
RS/DR4R3	4.2	0.004	5	0.013	23
RS/DR5R2	4.9	0.013	4	0.040	24
RS/DR7R	4.5	0.067	5	0.21	24
RS/RR	0.7	1.2	0.8	1.8	8.1

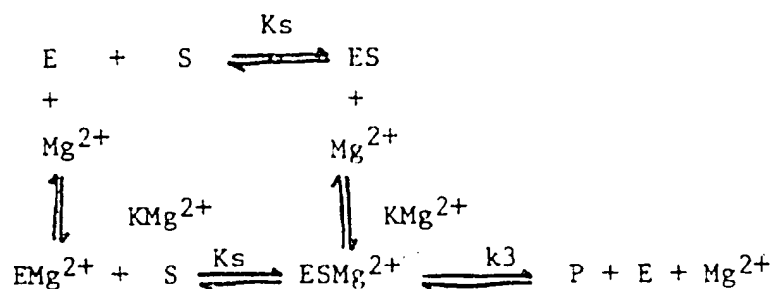[a] The kinetic experiments were performed under the
standard conditions of FIG 2 with following
differences: the final concentrations were:
nucleozyme 0.05 μM; substrate from 0.5 μM to 5
μM; Mg^{2+} from 5 to 50 mM and the reaction times
from 30 min to 6 h at 30°C. Initial rates were
determined from the first 10% of the reactions. The
chronology of substrate and metal ion addition is

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currently not known. The values for Ks, k3 and KMg
were, therefore, determined from the following random
assembly model for ternary complex formation:

The generalized reaction scheme used to
determine the K values is depicted below.

$$
\begin{array}{ccc}
\text{E} & + & \text{S} \xrightleftharpoons{\text{Ks}} \text{ES} \\
+ & & + \\
\text{Mg}^{2+} & & \text{Mg}^{2+} \\
\Big\updownarrow \text{KMg}^{2+} & & \Big\updownarrow \text{KMg}^{2+} \\
\text{EMg}^{2+} + \text{S} \xrightleftharpoons{\text{Ks}} \text{ESMg}^{2+} & \xrightleftharpoons{\text{k3}} & \text{P} + \text{E} + \text{Mg}^{2+}
\end{array}
$$

The reaction scheme starts with the catalytic
species, the substrate and the Mg^{+2} cofactor,
traversing a ternary complex and yielding a product.
The apparent K_m's and K_s's of the three
nucleozymes are virtually identical and approximately
five times those of the all-RNA ribozyme acting on an
RNA substrate.

The chimeric polymers were synthesized as
follows:

Ribonucleotide, Deoxyribonucleotide, or 2'-Methoxy
Analogue Phosphoramidite Synthesis and Purification

Dry N-Acyl-5'-O-DMT-2'-O-sily-ribonucleoside (10
mmol, 1 eq) was dissolved in 30 ml dry THF in a 300
ml round bottom flask. 2, 4, 6-Collidine (75 mmol,
7.5 eq) was added followed by N-methylimidazole (5
mmol, 0.5 eq). (N,N-dispropylamino) (cyanoethyl)
phosphonamidic chloride (20 mmol, 2.0 eq [22 mmol,

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2.2 eq in the case of guanosine or other nucleotide
bases with reactive lactams]) was then added dropwise
over 5 min at room temperature. The reaction was
clear at the start but a white precipitate formed
within 3 min (this is the collidine hydrochloride
salt). The reaction was complete after 1-2 hour
(determined by TLC). The reaction was then placed in
an ice bath and diluted with 100 ml (1 volume) ethyl
acetate. 150 ml 5% NaHCO$_3$ was added slowly (the
first 10 ml over 5 minutes). (In the case of
guanosine the reaction was first quenched with 5 ml
of absolute ethanol). Heat was generated by the
quenching of excess phosphitylating reagent. The
mixture was then transferred to a separatory funnel
and a second volume of ethyl acetate (100 ml) was
added after first rinsing out the reaction flask.
The aqueous phase was removed and the organic phase
washed with saturated NaCl. The combined aqueous
washes were back extracted with 50 ml. ethyl acetate
and the combined organic phases were dried over
Na$_2$SO$_4$. The solvent was removed in vacuo
yielding a viscous oil. Coevaporation (x 5) with 50
ml toluene afforded the crude phosphoramidite as an
offwhite foam or oil. Excess phosphinic acid and
collidine caused it to be oily. After leaving the
amidite under high vacuum overnight, resuspension and
rotovaping of the amidite with methylene chloride and
ethyl acetate usually produced a foam. The
phosphoramidites were further purified by silica gel
chromatography yielding a white foam in 75-85%

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yields. This procedure was repeated for the
preparation of the other desired phosphoramidites
changing the initial protected nucleotide unit. The
phosphoramidites were used to synthesize the mixed
polymers using automated synthesis techniques
commonly used for the synthesis of
deoxyribonucleotides.

Automated Synthesis of Mixed Polymers

All syntheses were conducted on either a Gene
Assembler Plus (Pharmacia), or a Cyclone
(Milligen/Biosearch) synthesizer using standard
protocols with an extended 12 min coupling step. A
30 fold excess (150 µl of 0.1 M = 15 mg, ~ 15
µmol) of the phosphoramidites and a 400 fold excess
of tetrazole (400 µl of 0.5M = 200 µmol)
relative to CPG bound 5'-hydroxyl was used in each
coupling cycle. Synthesis scale was 0.5 µmol.
Average coupling yields on the Gene Assembler Plus,
monitored by an on-line colorimeter, were ~99.0%
and on the Cyclone 97-98%, determined by colorimetric
quantitation of the trityl fractions. Reaction
columns for 0.5 µmol syntheses were
Milligen/Biosearch 1.0 µmol columns.
Oligonucleotide synthesis reagents: 1) for GA plus:
detritylation solution was 2% TCA in ethylene
dichloride; capping was performed with 20% N-Methyl
imidazoyl in THF and 10% acetic anhydride/10%
2,6-lutidine in THF; oxidation solution was 0.02
MI_2, 1% lutidine, 10% water in tHF. Baker

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Bio-Analyzed grade acetonitrile was further dried over activated 4 A molecular sieves. Tetrazole solution (0.5 M in acetonitrile was obtained from Applied Biosystems. 2) for Cyclone: all standard DNA synthesis ancillary reagents were used.

Deprotection of Mixed Polymers

The CPG-bound mixed polymer was transferred from the synthesis column to a 4 ml glass screw top vial. 1 ml of ethanolic ammonia was added and heated at 55°C for 16 hr. After cooling to -20°C, the ethanolic ammonia was removed from the CPG beads and the CPG was washed with 0.5 ml of 50:50/ethanol:water which was then added to the ethanolic ammonia. The combined supernatants containing the oligoribonucleotide were dried to a white powder. To remove the silyl protecting groups, the ammonia-deprotected mixed polymer was resuspended in 50 μl of 50:50/ethanol:water and 600 μl of 1M TBAF/THF and left at room temperature for about 24 hr. The solution was then added directly to 10 ml of 0.1M TEAB and loaded onto a Qiagen 500 anion exchange cartridge (Qiagen Inc., Studio City, CA) prewashed with 10 μl of 0.05M TEAB, the nucleozyme was eluted with 7 ml of 2M TEAB and dried down to a white powder.

Gel Purification of Fully Deprotected Mixed Polymers

The oligomers were first checked by analytical PAGE (0.75 mm x 20 cm x 45 cm). 1 ODU of oligonucleotide in 5 μl H_2O was added to 5 μl

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of deionized formamide and the total 10 μl solution
was loaded into a 1 cm wide lane. Following
electrophoresis the gels were photographed by placing
the gel over a fluorescent TLC plate and illuminating
the gel with a UV lamp. The desired sequence was
established according to electrophoretic mobility and
purified by preparative electrophoresis using 1.5 mm
thick gels with a single 12 cm wide lane. After
electrophoresis the desired band was excised,
crushed, placed into a sterile 5 ml test tube, and
covered with 50 mM NH$_4$OAc pH 7.0. The tube was
covered and kept at 37°C O/N. The supernatant was
then removed and the gel pieces washed with an
additional 1 ml of the extraction buffer. The
combined washings were filtered through a 0.45 micron
filter and loaded onto a 1 gram size Sep-Pak C18
cartridge (Waters-Millipore) prewashed with 5 ml each
of acetonitrile, 50% acetonitrile/0.1M TEAB and 0.1M
TEAB. After washing the cartridge with 5 ml of 0.1 M
TEAB, the RNA was eluted in 5 ml 35:35:30
acetonitrile/methanol/water and dried down to a white
powder.

The mixed polymers set forth in the Table of
FIG 2 were prepared as described above.

EQUIVALENTS

Those skilled in the art will be able to
ascertain, using no more than routine
experimentation, many equivalents of the specific
embodiments of the invention described herein.

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These and all other equivalents are intended to
be encompassed by the following claims.
What is claimed is:

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CLAIMS

1. A nucleozyme having catalytic activity, comprising:

a ribonucleotide and at least one second unit being a deoxyribonucleotide or nucleic acid analogue, the nucleozyme having a ribonucleotide or nucleic acid analogue at a catalytically critical site.

2. The nucleozyme as claimed in claim 1 including at least one deoxyribonucleotide.

3. The nucleozyme as claimed in claim 2 wherein all of the second units are deoxyribonucleotides.

4. A nucleozyme as claimed in claim 1 wherein the second units include at least one nucleic acid analogue.

5. A nucleozyme as claimed in claim 1 wherein the second units include at least 50% nucleic acid analogues.

6. A nucleozyme as claimed in claim 4 wherein the analogue contains a heterocylic ring.

7. A nucleozyme as claimed in claim 4 wherein the analogue is acyclic.

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8. A nucleozyme as claimed in claim 4 wherein
the analogue has the following formula:

$$HO \longrightarrow R_3 \quad B$$
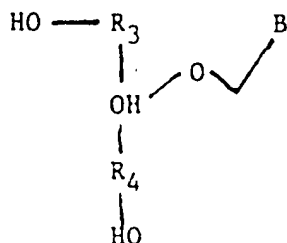
wherein B is a base and R_3 and R_4 are
each independently lower alkyl groups.

9. A nucleozyme as claimed in claim 8 wherein B
is selected from the group of adenine, cytosine,
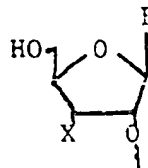guanine, uracil, 2-aminopurine,

hypoxanthine, and imidazoyl, $R_1\overset{\overset{\displaystyle O}{\|}}{C}-O-$, R_1-SH-,
R_1-NH_2- wherein R_1 is a lower alkyl group.

10. A nucleozyme as claimed in claim 4 wherein
the ribonucleotide analogue has a formula selected
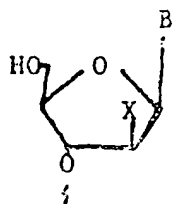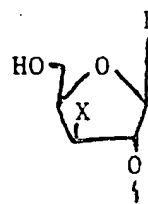from the group consisting of:
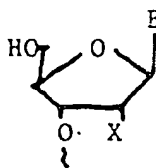
Formula (I)   Formula (III)

Formula (II)   Formula (IV)

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wherein B is a base and X is a good
coordinating ligand for a divalent metal ion.

11. A nucleozyme as claimed in claim 10 wherein
B is selected from the group consisting of adenine,
cytosine, guanine, uracil, 2-aminopurine,
hypoxanthine, and imidazoyl;
    and X is selected from the group consisting of
$-OR_1$, F, $-R_2OH$, and $-CH_2F$, wherein R_1 is a
lower alkyl group and R_2 is a lower alkyl chain.

12. A nucleozyme as claimed in claim 10 wherein
the analogue has a formula as shown in (I) and X is
methoxy.

13. A nucleozyme as claimed in claim 1 wherein
the nucleozyme has a ribonucleotide at a plurality of
catalytically critical sites.

14. A nucleozyme as claimed in claim 1 wherein
the nucleozyme has less than about 100 total building
units.

15. A nucleozyme as claimed in claim 14 wherein
the nucleozyme has less than about 80 total building
units.

16. A nucleozyme as claimed in claim 15 wherein
the nucleozyme has less than about 70 total building
units.

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17.   A nucleozyme as claimed in claim 16 wherein
the nucleozyme has less than about 50 total building
units.

18.   A nucleozyme as claimed in claim 1 wherein
the nucleozyme has a sequence corresponding to the
ribozyme selected from the group consisting of
hammerhead and hairpin.

19.   A nucleozyme as claimed in claim 18 wherein
the ribozyme is the hammerhead ribozyme.

20.   A nucleozyme as claimed in claim 19 wherein
the catalytically critical sites are the G9, G12,
A13, and A29 positions.

21.   A nucleozyme as claimed in claim 1 wherein
the nucleozyme is selected from the group consisting
of DR4R2, DR4R3, DR5R2, DR5R3, DR6R2, DR7R, MR5R2,
MR3R, and MR2R.

22.   A nucleozyme having catalytic activity,
comprising:
        a modified ribozyme having at least a
portion of its ribonucleotides replaced with
deoxyribonucleotides or nucleic acid analogs, the
modified ribozyme being significantly more resistant
to degradation than its all-RNA counterpart.

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23. A nucleozyme as claimed in claim 22 wherein
the resistance is to enzymatic degradation.

24. A nucleozyme as claimed in claim 22 wherein
the resistance is to chemical degradation.

25. A nucleozyme as claimed in claim 22 or 23
wherein the modified ribozyme is a modified
hammerhead ribozyme.

26. A nucleozyme as claimed in claim 22 wherein
the resistance is to enzymatic degradation and the
nucleozyme is at least 80% intact at an RNase A log
concentration of -2.5.

27. A nucleozyme as claimed in claim 26 wherein
the nucleozyme is at least 90% intact.

28. A nucleozyme as claimed in claim 22 wherein
the resistance is to enzymatic degradation and the
nucleozyme is at least 75% intact at an RNase A log
concentration of -1.5.

29. A nucleozyme as claimed in claim 28 wherein
the nucleozyme is at least 90% intact.

30. A nucleozyme as claimed in claim 25 wherein
the resistance is to enzymatic degradation and the
nucleozyme is at least 80% intact at an RNase A log
concentration of -2.5.

31. A nucleozyme as claimed in claim 30 wherein the nucleozyme is at least 90% intact.

32. A nucleozyme as claimed in claim 25 wherein the resistance is to enzymatic degradation and the nucleozyme is at least 75% intact and an RNase A log concentration of -1.5.

33. A nucleozyme as claimed in claim 32 wherein the nucleozyme is at least 90% intact.

34. A nucleozyme having catalytic activity comprising
    a chimeric polymer made up of RNA and second units being deoxyribonucleotides or nucleic acid analogues, said chimeric polymer having catalytic activity and having a majority of its total units being deoxyribonucleotides or nucleic acid analogs.

35. A nucleozyme as claimed in claim 34 wherein at least one of the second units is a deoxyribonucleotide.

36. A nucleozyme as claimed in claim 34 wherein all of the second units are deoxyribonucleotides.

37. A nucleozyme as claimed in claim 34 wherein the second units include a nucleic acid analogue.

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38. A nucleozyme as claimed in claim 37 wherein the second units include a plurality of nucleic acid analogues.

39. A nucleozyme as claimed in claim 37 wherein at least one of the nucleic acid analogues contains a heterocyclic ring.

40. A nucleozyme as claimed in claim 37 wherein the nucleic acid analogue is acyclic.

41. A nucleozyme as claimed in claim 38 wherein the analogue has the following formula:

$$
\begin{array}{c}
\text{HO} - R_3 \qquad B \\
| \qquad \diagup O \diagdown \\
\text{OH} \\
| \\
R_4 \\
| \\
\text{HO}
\end{array}
$$
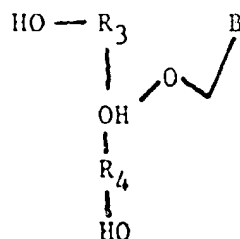
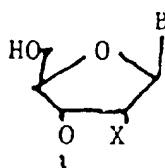wherein B is a base and R_3 and R_4 are each independently lower alkyl groups.

42. A nucleozyme as claimed in claim 39 wherein the base is selected from the group of adenine, cytosine, guanine, uracil, 2-aminopurine,

$$
\begin{array}{c}
O \\
\|
\end{array}
$$

hypoxanthine, and imidazoyl $R_1C\text{-}O\text{-}$, $R_1\text{-}SH\text{-}$, $R_1\text{-}NH_2\text{-}$ wherein R_1 is a lower alkyl group.
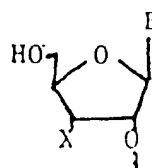
43. A nucleozyme as claimed in claim 37 wherein the analogue has a formula selected from the group consisting of:
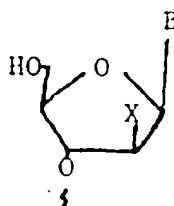
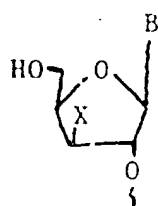Formula (I)   Formula (III)

Formula (II)   Formula (IV)

wherein B is a base and X is a good coordinating ligand with a divalent metal ion.

44. A nucleozyme as claimed in claim 40 wherein the base is selected from the group consisting of adenine, cytosine, guanine, uracil, 2-aminopurine, hypoxanthine, and imidazoyl;

and X is selected from the group consisting of $-OR_1$, F, $-R_2OH$, and $-CH_2F$, wherein R_1 is a lower alkyl group and R_2 is a lower alkyl chain.

45. A nucleozyme as claimed in claim 43 wherein the analogue has a formula as shown in (I) and X is methoxy.

46. A nucleozyme as claimed in claim 34 wherein all of the second units are 2'-methoxy ribonucleotide analogues.

47.   A nucleozyme as claimed in claim 34 wherein the nucleozyme is a modified hammerhead ribozyme.

48.   A nucleozyme as claimed in claim 34 or 47 wherein at least 50% of the polymer is second units.

49.   A nucleozyme as claimed in claim 48 wherein at least 75% of the polymer is second units.

50.   A nucleozyme as claimed in claim 49 wherein at least 85% of the polymer is second units.

51.   A nucleozyme as claimed in claim 50 wherein at least 90% of the polymer is second units.

52.   A chimeric polymer including at least one ribonucleotide and at least one second unit being a deoxyribonucleotide or nucleic acid analogue, the polymer having catalytic activity optimized by the presence of RNA at a catalytically critical site.

53.   A chimeric polymer as claimed in claim 52 wherein the second units include a deoxyribonucleotide.

54.   A chimeric polymer as claimed in claim 52 wherein RNA is present at all of the catalytically critical sites.

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55.   A chimeric polymer as claimed in claim 53
wherein all of the second units are
deoxyribonucleotides.

56.   A method of making a chimeric polymer
comprising:

        phosphitylating protected RNA or second
units under conditions to form substantially
pure-protected phosphoramidites of a single isomer,
the second units being deoxyribonucleotides or
nucleic acid analogues;

        coupling the protected phosphoramidites
together forming a protected chimeric polymer chain;
and

        deprotecting the chimeric polymer chain
under conditions which completely deprotect the
chimeric polymer.

57.   A method as claimed in claim 56 wherein the
second units in the phosphitylating step include a
deoxyribonucleotide.

58.   The method as claimed in claim 56 or 57
wherein the phosphitylating agent in the
phosphitylating step is (N,M-disopropyl amino)
(cyanoethyl) phosphonamidic chloride.

59.   A method as claimed in claim 56 or 57
wherein the phosphitylating step is conducted in the
presence of 2,4,6-collidine and N-methylimidazole.

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60.   A method as claimed in claim 58 wherein the
phosphitylating step is conducted in the presence of
2,4,6-collidine and N-methylimidazole.

61.   A method as claimed in claim 56 wherein the
deprotecting step is conducted in the presence of
ethanolic ammonia.

62.   A method as claimed in claim 59 wherein the
deprotecting step is conducted in the presence of
ethanolic ammonia.

63.   A chimeric polymer containing
ribonucleotide units and second units joined together
forming a chimeric polymer, the second units being at
least one deoxyribonucleotide or nucleic acid
analogue and the chimeric polymer being free of
protecting groups.

64.   A chimeric polymer as claimed in claim 63
wherein the second units include deoxyribonucleotides.

65.   A homogeneous RNA/DNA polymer containing
both deoxyribonucleotide and ribonucleotide units
joined together forming a mixed polymer, the mixed
polymer being free of undesired isomeric products.

66.   A homogeneous polymer as claimed in claim
65 wherein the undesired isomeric products contain
5-2 linkages.

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67.   In a chimeric nucleotide polymer synthesis,
the improvement comprising, the use of a catalyst
capable of minimizing migration of protecting groups
during a phosphitylation step.

68.   A method as claimed in claim 67 wherein the
catalyst is a combination of 2,4,6-collidine and
N-methylimidazole.

69.   In a chimeric nucleotide polymer synthesis,
the improvement comprising, the removal of protecting
groups from a protected mixed polymer in the presence
of ethanolic ammonia.

70.   A method for selectively cleaving an RNA
substrate, comprising:
        contacting a substrate with a nucleozyme
which targets a specific sequence in the substrate
for cleavage.

71.   A method for treating a subject for a
retrovirus associated disease, comprising:
        administering a therapeutically effective
amount of a nucleozyme to the subject such that the
nucleozyme cleaves the RNA genome of the retrovirus
or viral m RNA rendering it inactive.

72.   A method as claimed in claim 71 wherein a
plurality of nucleozymes are administered.

73.   A method as claimed in claim 71 wherein the
retrovirus is human immunodeficiency virus.

74.   A pharmaceutical composition, comprising:
       at least one nucleozyme; and
       a pharmaceutically acceptable carrier.

75.   A method of polymerizing an oligonucleotide
molecule comprising:
       contacting a template with a population of
nucleotide monomer units and a nucleozyme such that
an oligonucleotide complementary to the template is
formed.

76.   A method as claimed in claim 75 wherein the
oligonucleotide formed is an oligoribonucleotide.

77.   A method as claimed in claim 75 wherein the
oligonucleotide formed is an oligodeoxyribonucleotide.

78.   A method as claimed in claim 70 wherein the
RNA is messenger RNA.

79.   A method for controlling the expression of
a gene in a subject comprising:
       selecting a nucleozyme which targets an RNA
sequence involved in the expression of a gene;
       administering an effective amount of the
selected nucleozyme to the subject such that the
nucleozyme targets the RNA sequence and inhibits the
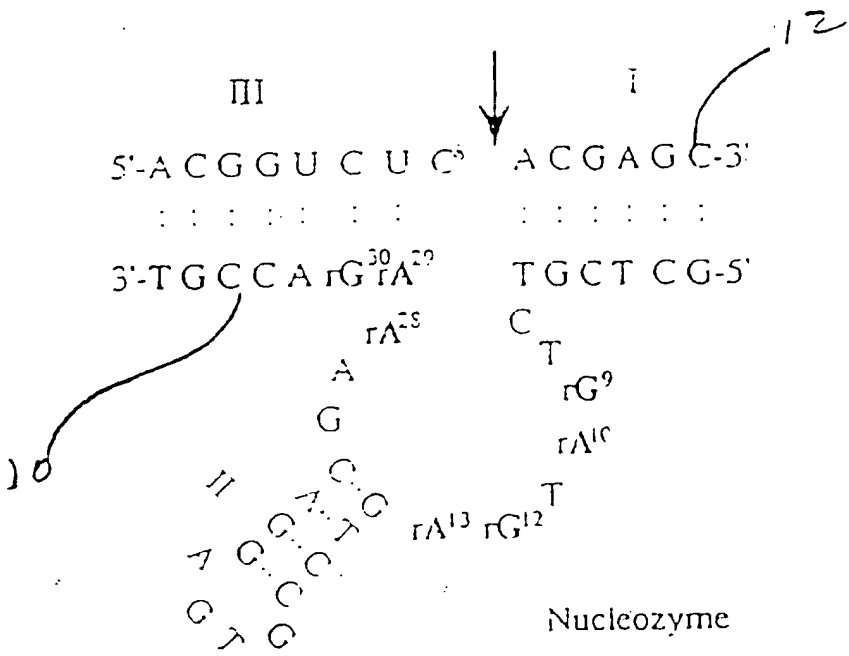expression of a gene.

FIG 1

| | Position of ribonucleotide | | |
	rG^9A^{10}	$rG^{12}A^{13}$	$rA^{28}A^{29}G^{30}$
Nucleozyme			
DR2R	rG	rA	- -
DR3R	rG	rA	rA
DR4R1	rG	rA	rArG
DR4R2	rG	rGrA	rG
DR4R3	rG	rGrA	rA
DR5R2	rG	rGrA	rArG
DR5R3	rGrA	rGrA	rG
DR6R2	rGrA	rGrA	rArG
DR7R	rGrA	rGrA	rArArG
OMe-nucleozyme			- -
MR2R	rG	rA	
MR3R	rG	rA	rA
MR5R	rG	rGrA	rArG

FIG 2

FIG 3

FIG 4

FIG 5

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FIG 6

FIG 7

I. CLASSIFICATION OF SUBJECT MATTER   (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N9/00;   A61K31/70;   C07H21/00;   C12P19/34

II. FIELDS SEARCHED

Minimum Documentation Searched[7]	
Classification System	Classification Symbols
Int.Cl. 5	C12N ; A61K ; C07H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[°]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,9 103 162 (CITY OF HOPE) 21 March 1991 see the whole document	1-3, 13-20 22-23, 25-36, 47-57, 63-66, 70-71 73-74, 78-79
Y		56-62, 67-69

	-/--	

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 MAY 1993	03. 06. 93
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	ANDRES S.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	NUCLEIC ACIDS RESEARCH vol. 18, no. 18, 25 September 1990, ARLINGTON, VIRGINIA US pages 5433 - 5441 SCARINGE, S.A. ET AL. 'Chemical synthesis of biologically active oligoribonucleotides using beta-cyanoethyl prtected ribonucleoside phosphoramidites' cited in the application	56-62
Y	see the whole document	56-62, 67-69

X	SCIENCE vol. 253, 19 July 1991, LANCASTER, PA US pages 314 - 317 PIEKEN, W.A. ET AL. 'Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes' see the whole document	1,4-6, 10-11, 13-20, 22-23, 25-34, 37-39 42-43, 47-48, 52,54, 56,63,70

X	BIOCHEMISTRY vol. 30, no. 16, 1991, EASTON, PA US pages 4020 - 4025 PERREAULT, J.-P. ET AL. 'Relationship between 2'-hydroxyls and magnesium binding in the hammerhead RNA domain: a model for ribozyme catalysis' cited in the application see the whole document	1-3, 13-20, 34-35, 47, 52-54, 63-66,70

X	WO,A,8 804 300 (UNIVERSITY PATENTS, INC.) 16 June 1988 see page 16, line 10 - page 17, line 24; claim 72	75-76

X	SCIENCE vol. 251, 20 March 1991, LANCASTER, PA US pages 1605 - 1608 DOUDNA, J.A. ET AL. 'A multisubunit ribozyme that is a catalyst of and template for complementary strand RNA synthesis' see the whole document	75-76

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	BIOCHEMISTRY vol. 31, no. 21, 2 June 1992, EASTON, PA US pages 5005 - 5009 YANG, J.-H. ET AL. 'Minimum ribonucleotide requirement for catalysis by the RNA hammerhead domain' see the whole document ---	1,4-6, 10-34, 37-39, 42-52, 54,56, 58-63 67-70
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P,X	WO,A,9 207 065 (MAX-PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.) 30 April 1992 see page 4, line 6 - page 7, line 3 see claims; example 4	1,4-6, 10-11 13-20 22-34, 37-39, 42-44 47-52, 54,63, 70-71, 73-79

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The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.   27/05/93

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		DE-T-	4091533	30-01-92
		EP-A-	0451221	16-10-91
		EP-A-	0506666	07-10-92
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		US-A-	5093246	03-03-92
WO-A-9207065	30-04-92	None		

(12) PATENT ABRIDGMENT   (11) Document No. AU-B-36001/93
(19) AUSTRALIAN PATENT OFFICE   (10) Acceptance No. 680405

(54) Title
NUCLEOZYMES

International Patent Classification(s)
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829729   31.01.92   **US UNITED STATES OF AMERICA**

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(57) Claim

1.   An enzymatic nucleic acid molecule having catalytic activity comprising a plurality of catalytically critical sites and a plurality of catalytically non-critical sites the enzymatic nucleic acid molecule including a catalytic centre comprising the catalytically critical sites, and a plurality of catalytically non-critical sites, wherein the catalytically critical sites are ribonucleotides or nucleic acid analogues capable of participating in the catalytic activity of the enzymatic nucleic acid molecule and a majority of the catalytically non-critical sites in the catalytic centre are deoxyribonucleotides, the enzymatic nucleic acid molecule having an increased resistance to degradation compared to an all RNA counterpart.